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Title : Involvement of D1 dopamine receptor in MDMA-induced locomotor activity and striatal gene expression in mice.

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA), a widely used recreational drug with psychoactive properties, induces both serotonin and dopamine release in the brain. In rats and mice MDMA induces behavioural changes and has rewarding effects but little is known about its cellular effects. We have previously shown that the ERK pathway is important for the changes in gene expression observed in mice striatum after treatment with this psychostimulant. In this study we investigated the role of D1 receptors in MDMA-induced locomotor hyperactivity and regulation of immediate early genes (*Fos*, *Fosb*, *Egr1* and *Egr2*) mRNA levels requiring ERK activity in mice striatum. We used the selective D1 receptor antagonist, SCH23390 at a dose (0.05mg/kg) that did not influence locomotor activity. This dose totally blocked MDMA-induced locomotor activity but only partially the increase in transcription levels of *Fos*, *Fosb*, *Egr1* and *Egr2* (24%, 23%, 22% and 29% respectively). In conclusion, our results showed that D1 receptors play a key role in the acute MDMA-induced hyperlocomotion and that ERK pathway is partially under D1 receptors control to induce *Fos*, *FosB*, *Egr1* and *Egr2* transcription.

Section 1 : Cellular and Molecular Biology of Nervous Systems

Key words : MDMA; D1 receptor; locomotor activity; ERK; immediate-early genes.

Abbreviations : MDMA, 3,4-Methylenedioxymethamphetamine. IEGS, immediate-early genes. DA, dopamine. ERK, extracellular signal-regulated kinase. Hpvt, hypoxanthine guanine phosphoribosyl transferase.

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is a popular recreational drug widely abused among young people. In rats and mice MDMA induces locomotor hyperactivity, hyperthermia, and has rewarding effects (for a review, see: Green et al., 2003). Furthermore, MDMA has been shown to be neurotoxic to serotonin terminals in rats and primates (Green et al., 2003). Despite the increasing abuse rate (Pope Jr. et al. 2001) and neurotoxic effects (Parrott 2002; Green et al., 2003) of MDMA, relatively few studies have attempted to characterize the mechanisms underlying its behavioural effects.

MDMA increases synaptic levels of monoamines, including dopamine (DA) primarily by acting as a substrate of monoamine re-uptake transporters (McCreary et al., 1999; Yamamoto and Spanos 1998). It is widely believed that increased DA transmission in striatum underlies the hyperlocomotor effects of amphetamine-like stimulants. Supporting a role for DA in these responses, behavioural studies have shown that blockade of dopamine D1 and D2 receptors significantly attenuates MDMA-induced locomotor activity in rats (Ball et al., 2003). Moreover, MDMA-induced enhancement of immediate early genes (IEGs), *Fos* and *Egr1* in rat striatum were inhibited by selective D1 and D2 receptor antagonists (Dragunow et al. 1991; Hashimoto et al., 1998; Shirayama et al. 2000).

Previous studies in the laboratory have shown that ERK (extracellular signal-regulated kinase) signalling is involved in several MDMA behavioural and transcriptional effects in mice, by using a specific inhibitor of ERK activation, SL327 (Salzmann et al., 2003). This inhibitor blocked MDMA hyperactive effects and reversed MDMA-induced enhancement of IEGs transcription in the dorsal striatum (Salzmann et al., 2003). Interestingly, ERK can be activated by several drugs of abuse (Valjent et al. 2004). In addition it was shown that psychostimulants activate ERK pathway within a restricted subpopulation of dynorphin and D1 receptor-positive striatal medium-sized spiny neurons in mouse (Valjent et al. 2005).

To further understand the importance of D1 receptors activation in the mechanisms underlying the striatal responses we used the D1 receptor antagonist, SCH23390. The effects of the antagonist on MDMA-induced locomotor hyperactivity and regulation of four IEGs under the controlled of ERK pathway (*Fos*, *Fosb*, *Egr1* and *Egr2*) were investigated in CD1 mice striatum.

2. Results

Dose-response curve of SCH23390 on locomotor activity

SCH23390 at different doses (0.015, 0.05, 0.20 and 0.7 mg/kg) seems produced U-shaped curve in affecting the locomotion of animals. SCH23390 at the doses 0.015, 0.05 and 0.7 mg/kg i.p. were ineffective in modifying the locomotor activity of mice, while SCH23390 at 0.2 mg/kg significantly decreased the locomotor activity as compared to control group (Figure1a).

SCH23390 (0.05 mg/kg) was chosen for further experiments as this dose did not induce significant modification of locomotor activity of mice.

Effect of SCH23390 on MDMA-induced locomotor activity

To examine whether blockade of D1 receptor would prevent development of MDMA induced locomotor activity, SCH23390 (0.05mg/kg) was injected 30 min before MDMA (9mg/kg). As compared to the saline treated mice, acute treatment with MDMA led to a significant increase of locomotor activity which was totally blocked by pre-administration of the D1 antagonist SCH23390 (Figure1b).

Effect of SCH23390 on the MDMA-induced immediate early genes expression

Real time quantitative PCR was used to measure the effect of the antagonist on the expression of immediate early genes after MDMA injection. The regulation of four genes known to be modulated by acute MDMA was studied: *Fos*, *Fosb*, *Egr1* and *Egr2*. As compared to the saline-treated mice, acute treatment with MDMA led to a significant increase in *Fos* and *FosB* mRNA in the striatum (5.7 and 6.7 fold respectively). Acute MDMA treatment also induced a significant increase in *Egr1* and *Egr2* mRNA (2.3 and 4.2 fold respectively). The pre-injection of the D1 receptor antagonist partially, but significantly, blocked the increased transcription of *Fos*, *FosB*, *Egr1* and *Egr2* (24%, 23%, 22% and 29% respectively)(Figure2).

3. Discussion

The aim of the present study was to characterize the role of D1 receptors in MDMA-induced locomotor hyperactivity and regulation of IEGs expression requiring ERK activity in mice striatum.

We found that the selective D1 antagonist receptor, SCH23390 produced a U-dose response curve on mice locomotor activity. A dose of SCH23390 that did not influence locomotor activity was chosen (0.05 mg/kg). This dose blocked MDMA-induced hyperactivity in CD1 mice demonstrating that D1 receptors are involved in MDMA-induced locomotor activity. This result is in agreement with previous studies performed in rats showing that SCH23390 was able to antagonize the acute behavioural responses induced by MDMA (Ball et al., 2003). In previous studies we have also shown that ERK pathway plays a role in MDMA-induced locomotor activity in mice (Salzmann et al. 2003). Together these results suggest that the acute MDMA-induced hyperlocomotion is very likely mediated via the ERK pathway subsequent to D1 receptors activation.

ERK is an important regulator of neuronal functions, and is involved in various neurobiological events such as synaptic plasticity and memory (see Review in Sweatt, 2001). It has been suggested that ERK may be a common pathway to different drugs of abuse and plays a role in rewarding properties of MDMA, THC and cocaine (Salzmann et al. 2006; Valjent et al. 2001). In addition it has been shown that *d*-amphetamine- and cocaine- activated ERK pathway was restricted within a subpopulation of dynorphin and D1 receptor-positive striatal medium-sized spiny neurons in mouse (Valjent et al. 2005). Moreover, recently Acquas et al. (2007) have shown that activated ERK may represent a post-synaptic correlate of the stimulant effect of MDMA on D1-dependent dopamine transmission in the ventral striatum of rat.

In a previous study it was demonstrated that ERK activation was involved not only in the behavioural effects of MDMA but also in its transcriptional effects on IEGs belonging to the Fos and Egr families (Salzmann et al. 2006). Interestingly while the locomotor effects induced by MDMA were totally blocked by SCH23390, MDMA-induced transcriptional activation of two members of the Fos gene family was only partially prevented by SCH23390. This suggests that D1 receptors are probably not the only pathway leading to this regulation. These results are consistent with those from Salzmann et al. (2006) showing that MDMA-induced transcriptional activation of Fos and Fosb were partially prevented by SL327 treatment. This suggests that the regulation of these genes by acute MDMA treatment was mediated by

several signalling pathways among which one was dependent on ERK activity. The same authors showed using real time quantitative PCR, that MDMA-induced *Egr1* and *Egr2* transcriptions were totally dependent upon ERK activation, while we demonstrated that these effects were only partially dependent upon D1 receptors activation. Our finding appears in good agreement with studies conducted in rat striatum showing that *Fos* and *Egr1* expression induced by MDMA were inhibited by pretreatment with SCH23390 (Draunow et al., 1991; Hashimoto et al., 1998; Shirayama et al., 2000). An implication of NMDA receptors and serotonin transporters have also been suggested (Shirayama et al., 2000). All together these results suggest that D1 receptors are not the only receptors activated by MDMA that could lead to ERK activation inducing rapid and transient expression of several IEGs. One hypothesis could be that serotonin receptor located on dynorphin and D1 receptor positive striatal neurons may contribute to the activation of ERK pathway. Indeed several studies have shown that serotonin receptors are present on striatonigral neurons that express preprodynorphin mRNA, and that MDMA may increase the extracellular concentration of serotonin in the striatum (Valjent et al. 2005).

In conclusion, our results showed that D1 receptors play a key role in the acute effects of MDMA-induced hyperlocomotion and that the ERK pathway is partially under D1 receptors control to induce *Fos*, *FosB*, *Egr1* and *Egr2* transcription.

4. Experimental Procedure

Animals and Drugs

Male CD-1 mice (Charles River, L'Arbresle, France) weighing 22–24g were housed in a room with an alternating 12-h light/dark cycle and a controlled temperature ($21\pm1^{\circ}\text{C}$). Food and water were available *ad libitum*. Care and treatment of animals conformed to the ethical standards and guidelines promulgated by the European Communities Council Directive (86/609/EEC). All efforts were made to minimize animal sufferings and to use only the number of animals necessary to produce reliable scientific data.

All drugs were injected i.p., D,L-MDMA (Lipomed, Arlesheim, Switzerland) and R (+)-SCH23390 (Sigma, France) were dissolved in saline 0.9%. In all cases the injection was 0.1ml/10g body weight.

Locomotor activity

The locomotor activity of mice was measured in an actimeter composed of eight cages of transparent plastic of equal size (19x11x14 cm) under low illumination (5 lux). Displacements were measured by photocell beams located across the long axis, 20 mm (horizontal activity) above the floor (Immetronic, Bordeaux, France). In a first experiment dose response curve of SCH23390 was performed. SCH23390 (0.015, 0.05, 0.20 and 0.7 mg/kg) was i.p. injected and 30 min after animals was placed in the actimeter. In a second experiment, MDMA (9 mg/kg) (as previously described Salzmänn et al, 2003) or saline was injected 30 min after an inactive dose of SCH23390 (0.05mg/kg) or saline, and mice were immediately placed in the actimeter. Locomotion activity was recorded for 60 min and expressed in scores (mean \pm S.E.M.) as the total number of interruption of the photocell beams.

Drug treatments and dissection for RT-PCR analysis

Mice were injected with SCH23390 0.05 mg/kg (or saline) 30 min before MDMA 9mg/kg (or saline) injection. All animals received two injections. For transcriptional studies mice were killed by cervical dislocation 2 hours after the last injection. The brain was quickly removed, frozen in isopentane at -50°C , and placed in an acrylic matrix (David Kopf Instruments, Tujunga, CA, USA) allowing the reproducible slicing of 1mm coronal sections. A section of 2mm was cut, corresponding approximately to bregma +0.26mm to -0.46mm according to the mouse brain atlas (Academic Press, 2nd edition, Paxinos and Franklin, 2001). Dorsal striatum was then dissected free-hand on ice within the slice, and stored at -80°C until processing.

RNA isolation and Reverse Transcription for quantitative PCR

Total RNA used for quantitative PCR experiments were extracted by a modified acid-phenol guanidinium method, following the manufacturer's protocol (RNABle®, Eurobio, France). The quality of the RNA samples was determined by electrophoresis through agarose gels and staining with ethidium bromide. Quantification of total RNA was assessed using a NanoDrop® ND-1000 spectrophotometer (NanoDrop® Technologies, USA). Reverse transcription of RNA was performed in a final volume of 20 μl containing 1x first strand buffer (Invitrogen, France), 500 μM each dNTP, 20 U of Rnasin ribonuclease inhibitor (Promega, France), 10 mM dithiothreitol, 100 U of Superscript II Rnase H⁻ reverse transcriptase (Invitrogen, France), 1.5 μM random hexanucleotide primers (Amersham Biosciences, France) and 1 μg of total RNA.

Real-time quantitative RT-PCR

PCR primers were chosen with the assistance of Oligo 6.42 software (MedProbe, Norway). The primer nucleotide sequences used for *Hprt* (hypoxanthine guanine phosphoribosyl transferase), *Fos*, *FosB*, *Egr1* and *Egr2* have been previously described (Salzmann et al, 2003; Salzmann et al 2006). Fluorescent PCR reactions were performed on a 7900HT system (Applied Biosystems, France) using the Power SYBR® Green PCR Master mix kit (Applied Biosystems, France). The cDNAs were diluted 200-fold and 8 µl were added to the PCR reaction mix to yield a total volume of 20 µl. The reaction buffer contained 0.5 µM of each primer. The PCR reactions were performed with 10 samples/drug treatment, each sample being prepared with bilateral structures from one mouse. Experiments were performed in duplicates for each data point. Quantification was made on the basis of a calibration curve using cDNA from an untreated mouse brain. As previously described, in addition to the genes of interest, the *Hprt* transcript was also quantified and each sample was normalized on the basis of its *Hprt* content (Salzmann et al, 2006). Results are expressed as gene of interest transcript / *Hprt* transcript.

Statistical analysis

Data were analyzed using one-way ANOVA between subjects for counts of locomotor activity and quantitative RT-PCR. Post hoc comparisons were made using the Fisher-PLSD test. The level of significance was set at $P < 0.05$.

Figure Legends

Figure 1: (a) Effect of SCH23390 (0.015, 0.05, 0.2, and 0.7 mg/kg) on locomotor activity. The mice received SCH23390 (0.015, 0.05, 0.2, and 0.7 mg/kg) or saline i.p. 30min before the monitoring of the locomotor activity. (b) Effect of pretreatment with the D1 receptor antagonist SCH23390 (0.05mg/kg, i.p.) on MDMA (9 mg/kg, i.p.)-induced hyperlocomotor activity. The mice received SCH23390 or saline 30 min before MDMA or saline and the locomotor activity was measured during 60 min. Data were expressed as means \pm s.e.m. of cumulative number of interruption of photocell beams measured during 60 min (n= 10/12 mice). Statistical analysis was done by ANOVA, followed by Fisher's PLSD. * <0.05 and *** <0.001 as compared to control group; ### <0.001 as compared to SCH23390 and MDMA-treated group (Fisher's PLSD).

Figure 2: Real time quantitative PCR analysis of SCH23390 \pm MDMA treatment effect on Fos, FosB, Egr1 and Egr2 transcription. SCH23390 was administered 30 min before MDMA and mice were killed 2h after the last injection. The mRNA level was measured using quantitative real-time PCR and normalized to hrpt mRNA levels. Values represent means \pm s.e.m. (percentage of vehicle-treated animals; n= 12/group). Statistical analysis was done by ANOVA, followed by Fisher's PLSD. * <0.05 , ** <0.01 , *** <0.001 as compared to control group; # <0.05 , ## <0.01 as compared to SCH23390 and MDMA-treated group.

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